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Characterization of Compression Molded Sodium Caseinate-based Films

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CHARACTERIZATION OF COMPRESSION MOLDED SODIUM CASEINATE-BASED FILM

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ABSTRACT

The objective of this research was to characterize compression molded sodium caseinate based films and investigate the effect of water and glycerol ratios on thermal transition and heal sealing properties. Studies were conducted on films from three variations of film-forming mixtures or "resins", derived from US patent 4,076,846 containing sodium caseinate (NaCas), methylcellulose (MC), calcium chloride ($CaCl_2$), glycerol (Gly), and distilled water.

Moisture sorption isotherms were used to evaluate the water-protein and water-glycerol interactions in the resins. Sorption isotherms were characteristic of most biopolymer material and showed increasing moisture sorption with increasing amounts of glycerol. In compression molded films containing ≥29% glycerol, visible opaque specks of unincorporated matter were attributed to the high water binding property of glycerol and its interaction with the NaCas, which prevented the NaCas from being able to fully solubilize.

Thermal analysis by DSC and TMA showed that all films were amorphous and that water and glycerol were equally effective at lowering T_g within the different water: glycerol ratios that were tested. While water and glycerol were equally effective at lowering T_g, they affected the strength properties and heat seal strengths of the film in different ways. Increasing amounts of glycerol decreased heat seal strengths (maximum load force/width) at higher seal temperatures (195°F) because it interacted with the protein chains, increasing mobility and elongation of the film under tensile stress and

interfering with protein entanglements at the seal interface. Less glycerol increased stiffness causing increased film strength and seals with higher breaking stress.

DEDICATION

This thesis is dedicated to my loving husband, Ron, and to my boys, Nathan and Conner, and to my parents, Roger and Lynn Parish. Thank you for your endless support and encouragement throughout this entire journey. This is also dedicated to my beloved sister, Lesley, who always encouraged me to do more than I ever felt capable of doing and cheered me on.

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CHAPTER 1

INTRODUCTION

The rising cost of petroleum, combined with pressure to demonstrate packaging and product sustainability, is promoting research on bio-based material from renewable resources that are locally available. Milk proteins are commercially available and have been used for decades in a wide variety of industrial applications.

Sodium caseinate is a water-soluble polymer obtained by acid precipitation of casein, the main protein in bovine milk. Sodium caseinate has thermoplastic and film forming properties (transparency, biodegradability, good barrier to O_2, CO_2) that make it an interesting raw material for several applications in packaging. However, for a protein-based film to be considered commercially viable, it must be able to be produced in a continuous process like extrusion.

While most research has been conducted on solution cast sodium caseinate film, compression molding is a better precursor to extrusion in order to demonstrate material flow properties and facilitate molecular changes associated with heat and pressure. Several studies have been conducted on compression molded biopolymer films including wheat gluten films (Zuo, Song, & Zhenge, 2009) as well as whey protein films (Sothornvit, Olsen, McHugh, & Krochta, 2003). However, research is extremely scarce on sodium caseinate films formed by compression molding.

The aim of this research is to characterize compression molded sodium caseinate based films and investigate the effect of water and glycerol ratios on thermal transitions and heal sealing properties.

CHAPTER 2

LITERATURE REVIEW

Sustainability

In 1987 at the UN World Commission on Environment and Development, a definition for sustainability was created: Development that meets the needs of the present without compromising the ability of future generations to meet their own needs (Beczner, Gower, Katz, & Shedroff). With an ever-increasing global concern for the environment, every industry is being challenged to assess the sustainability of its products. One area of concern for the plastic industry involves the availability of the supply of raw materials from which they are produced. The majority of plastics are made from petroleum (crude oil), natural gas, and coal. These are natural resources that have taken thousands of years to be formed, and are considered nonrenewable. According to E.S. Stevens (2002), worldwide reserves for petroleum have been estimated to be in the range of 200 billion tons, which is only enough for approximately 50 years at the current rate of consumption. Some people feel that the use of limited fossil resources for the large-scale plastics industry is a legitimate environmental concern now (Stevens, 2002).

While availability of petroleum based raw materials is a concern, the foremost concern for the plastics industry and consequently the packaging industry is the rising cost of the raw materials. For countries like the United States that import the majority of

its oil, the economic burden of world oil prices is a driving factor for concerted efforts to evaluate the use of alternative materials.

Renewable Resources

The rising cost of petroleum, combined with pressure to demonstrate packaging and product sustainability, is promoting research on bio-based material from renewable resources that are locally available. In the Merriam-Webster dictionary, renewable is defined as "capable of being replaced by natural, ecological cycles or sound management practices" (www.merriam-webster.com). Most notably solar energy, water, air and trees are all considered renewable resources. The Sustainable Packaging Coalition declares: "Renewable materials are derived from biological systems and can be biodegraded and readily re-incorporated into the biosphere where they provide nutrients for a new cycle of production." When discussing packaging materials, those considered renewable are generally fiber and other bio-based materials that can be produced from annually renewable agricultural products such as corn or cane sugar.

Bio-based materials from various agricultural resources have been used throughout history and were significantly commercialized in the nineteenth century. Celluloid made from cellulose nitrate and camphor was the first widely applied plastic used to produce items such as combs, shoehorns, letter openers, photographic film, and windshields (Stevens, 2002).

Among other agricultural products, proteins have long been used as raw materials for plastic articles. Casein from milk was widely used commercially for paints, glues, and plastics. Soy meal plastics were developed by Henry Ford in the 1940's and used to make automobile parts like glove-box doors, gearshift knobs, steering wheels and eventually a complete prototype car with a soybean plastic body (Stevens, 2002).

Proteins

Proteins, made up of amino acids, are natural polymers existing in all living cells of animals and plants. Therefore, proteins are a renewable, biodegradable resource with great potential for a variety of applications. Today numerous cereal and vegetable proteins (such as corn zein, wheat gluten, and soy proteins) and animal proteins (such as milk proteins, collagen, gelatin, keratin, and myofibrillar proteins) are used as raw materials to form packaging materials (Cuq, Gontard, & Guilbert, 1998). Proteins are good film formers demonstrating good oxygen, carbon dioxide, and lipid barrier properties, particularly at low relative humidity (Lacroix & Cooksey, 2005).

Unlike some polysaccharides, which are often homopolymers constituted of one repeated monomer, proteins are heteropolymers composed of various combinations of more than 20 different amino acids. The almost limitless number of side-chain amino-acid sequential arrangements in proteins allows a wide range of interactions and chemical reactions to occur in thermal processing (Hernandez-Izquierdo & Krochta, 2008). The

result is production of materials with functional properties that are not possible with polysaccharides like starch and cellulose based materials alone.

Proteins have been used for centuries for a wide variety of applications, but the understanding of precise physical and chemical mechanisms of protein interactions continues to evolve (Hernandez-Izquierdo & Krochta, 2008). The functional properties of these materials depend on the protein's molecular structure, thermal sensitivity, and hydrophilic behavior (Cuq et al., 1998).

Casein

Because of their availability and numerous functional properties, milk proteins have been studied closely since the early 1800's (Fox & Kelly, 2004). The proteins that precipitate into a gel (curd) at pH 4.6 and 20°C are called caseins. The proteins that remain water soluble at pH 4.6 are known as whey proteins. Caseins represent 80% of the total protein found in milk from cattle and other dairy species (Fox & Brodkorb, 2008). Caseins in milk are large colloidal particles containing calcium phosphate, 50-600nm in diameter (mean 150nm), and are referred to as "casein micelles"(Fox & Brodkorb, 2008).

Acid and rennet caseins are the two major types of casein available (Kinsella, 1984). Commercially produced acid casein refers to casein precipitated from skim milk at its isoelectric point of pH 4.6. The destabilization and coagulation of casein by

enzymes that target proteins (proteolytic enzymes), like chymosin (rennin) or pepsin is used to create rennet casein.

Due to the ease of producing casein by isoelectric precipitation or rennet-induced coagulation, casein has been produced commercially since the early 20th century (Fox & Kelly, 2004). Initially, casein was used for only industrial applications like glues and paper glazing, then rigid plastics and textile fibers (J. Audic, Chaufer, & Daufin, 2003). In the 1960's the work done on casein in New Zealand and Australia led to its use as a functional food ingredient (Fox & Kelly, 2004). Technology for the production of acid and rennet caseins is well established and continues to improve.

Physical and Chemical Properties of Caseins

The caseins are small proteins with molecular weights between 20 and 25 kDa, (Fox & Kelly, 2004). The caseins exist as highly hydrated spherical micellar aggregates containing from 2 to 3 g of water per gram of protein (Kinsella, 1984). Bovine casein consists of four principle components: α_{s1}-, α_{s2}-, β- and κ- representing approximately 38, 10, 36, and 12%, respectively, of whole casein (Fox & Kelly, 2004). Although the components of the casein micelles are known, the precise microstructure of the subunits, as well as the micelle has not been agreed upon (Brunner, 1976). The calcium sensitive caseins α_{s1}-, α_{s2}-, and β- are thought to be predominately in the interior of the micelle while the surface of the micelle is covered with a layer of κ- casein (deKruif & Holt, 2003). Another theory suggests that each micelle is made up of submicellar particles (10

to 20 nm) held in the micelle matrix by colloidal calcium phosphate (CCP) (Kinsella, 1984). Figure 2.1 illustrates both theories of the casein micelle structure.

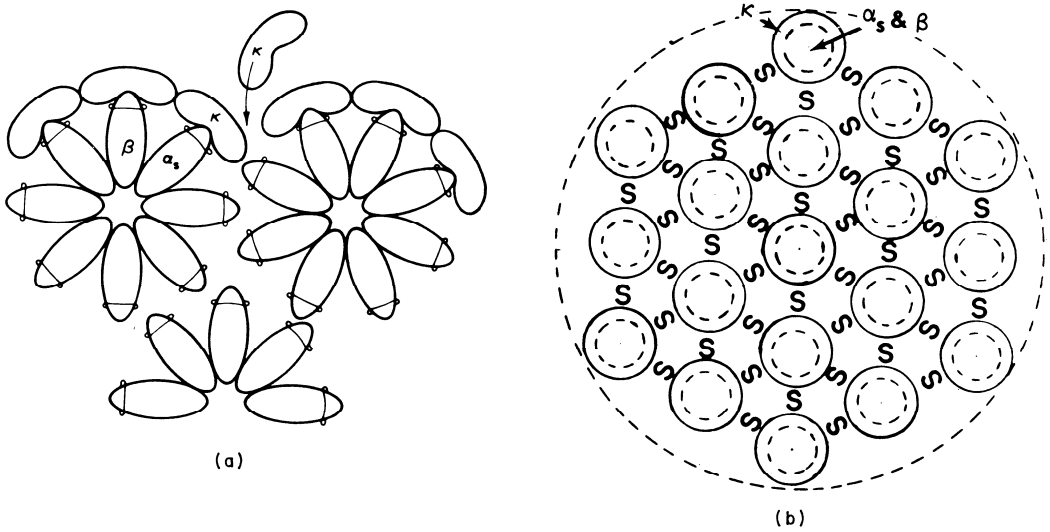

Figure 2.1 Two Conceptual Models of Micellular Casein (Brunner, 1976)
(S represents CCP)

All of the caseins are phosphorylated so they show avid calcium binding properties, are sensitive to pH, and possess hydrophilic and hydrophobic segments (Kinsella, 1984). The high levels of proline (an amino acid), especially in β-casein, contribute to their high surface activity for good foaming and emulsifying properties (Fox & Kelly, 2004).

Caseinates

Acid and rennet caseins manufactured by the processes described above are insoluble in water. Addition of alkali (such as sodium hydroxide, potassium hydroxide, calcium hydroxide, or ammonium hydroxide) causes the fresh curd or rehydrated casein to dissolve forming soluble protein products known as caseinates (Southward, 1985). The resulting caseinate solution can be dried by a spray or roller process to produce a caseinate powder.

Sodium Caseinate

Commercially available sodium caseinate is most commonly produced by dissolution of acid casein in sodium hydroxide at a pH close to 7 (J. Audic et al., 2003). Other alkalis, such as sodium bicarbonate or sodium phosphate, may be used but the amounts required and their costs result in higher total cost than that of sodium hydroxide (Southward, 1985).

Colloid mills are required to reduce the particle size of curd for rapid dissolution. The caseinate solution must be heated and the pH and viscosity carefully controlled during the dissolving operation. Dissolving vats must be equipped with powerful agitators and high-speed re-circulating pumps because sodium caseinate solutions of moderate concentration are extremely viscous (Southward, 1985). Addition of dilute alkali into the casein slurry is monitored to reach a final caseinate pH of 6.6-7.0.

Physical and Chemical Properties of Sodium Caseinate

Casein micelles are remarkably stable to heat and to physical forces. However the acid and alkaline treatments used to precipitate the casein disrupt the native structure of the casein particles therefore caseinates have little similarity to the native casein micelles in milk (Kinsella, 1984). The adjustment of pH to 4.5 dissociates the colloidal phosphate structure and frees the casein precipitate of calcium. Micelles identical to those found in milk probably do not exist at < pH 5 owing to the dissolution of colloidal calcium phosphate and perhaps other factors (Fox & Kelly, 2004). Whereas, at neutral pH, the casein micelles have been found to behave as hard spheres (Fox & Kelly, 2004), it has been suggested (de Kruif, 1998) that destabilization by acidification changes their behavior to that of "sticky" spheres. Stickiness and viscosity as well as film-forming ability, explain why caseinate solutions are used in the manufacture of glues, paper coating and sizing, films and biomaterials (J. Audic et al., 2003).

The alkaline treatment used to make sodium caseinate solubilizes the caseins by altering the net charge, overcoming hydrophobic interactions (Kinsella, 1984). Research suggests that the resulting caseinate aggregates are smaller and more sensitive to pH and ionic strength than the colloidal, phosphate-containing casein micelles in milk (Kinsella, 1984). Southward (1985) found water absorption of sodium caseinate to be > 200%, while that of casein and insoluble co-precipitates was <100%.

Sodium caseinate is highly water soluble above pH 5.5 and can be dispersed rapidly in aqueous mixtures and homogenized in the presence of fat or oil (Kinsella, 1984). Sodium caseinate is heat stable (140°C for 15 min at pH 7) (Kinsella, 1984). Jahaniaval et al. (2000) reported that this protein undergoes structural changes at pH 3.5 to 4 following heat treatment. At 132°C its viscosity, foaming and emulsifying capacity were significantly reduced but its foam stability appeared to improve (Guo, Fox, Flynn, & Kindstedt, 1996). Barreto et al. (2003) reported that thermogravimetric testing of sodium caseinate film made with 10% w/w of the protein in distilled water showed protein degradation between 295 and 300°C.

Typical functions of sodium caseinate in foods include water binding, fat binding, gelation, viscosity, and emulsion formation (Southward, 1985). Due to their high amount of polar groups, caseinate shows good adhesion to different substrates and this hydrophilicity makes caseinate films excellent barriers to non-polar substances such as oxygen, carbon dioxide, as well as non-polar aromas (J. Audic et al., 2003). Considering these properties, use of caseinate-based polymers for consumer products applications such as protective coatings, adhesives, films and packaging are of great interest in the field of biodegradable and environmentally friendly materials (J. Audic et al., 2003).

Sodium Caseinate Films

Sodium caseinate can easily form films from aqueous solutions because of its random coil nature and its ability to form extensive intermolecular hydrogen,

electrostatic, and possibly hydrophobic bonds (Khwaldia, Banon, Perez, & Desobry, 2004), (Gennadios, McHugh, Weller, & Krochta, 1994). Considering their transparency, biodegradability, good barrier properties (for O_2, CO_2, and aromas), caseinate based films can find applications in packaging in edible films, protective films and coatings, or in mulching films (J. -. Audic & Chaufer, 2005). Caseinate films are already used as microencapsulating agents of flavors and medicaments, in coatings of fruits, vegetables, and cheese (Khwaldia, Banon, Perez et al., 2004).

Improvement of Protein-based Film Properties

Most studies on sodium caseinate based films have focused on improving mechanical properties and/or reducing water vapor transmission rates. Most research has focused on addition of plasticizer (Barreto, Pires, & Soldi, 2003), (Khwaldia, Banon, Perez et al., 2004), (Siew, Heilmann, Easteal, & Cooney, 1999), incorporation of other proteins, lipids, and polysaccharides (Fabra, Talens, & Chiralt, 2008), (Arvanitoyannis & Biliaderis, 1998), (Kristo & Biliaderis, 2006), (Longares, Monahan, O'Riordan, & O'Sullivan, 2005) as well as addition of chemical crosslinkers (J. -. Audic & Chaufer, 2005).

Plasticizers

Protein-based films are brittle and require the addition of plasticizers to the protein matrix to improve processability and modify the properties of the final material.

Plasticizers are low molecular weight, low volatility substances that interact with polymer chains to increase free volume and mobility of molecules (Hernandez-Izquierdo & Krochta, 2008). Pommet and others (2005) tested several compounds with different chemical functions, different number of functional groups, and various degree of hydrophobicity as wheat gluten plasticizers. The critical factors for a good plasticizer were found to be low melting point, low volatility, and protein compatibility. Other considerations should include the amount of plasticizer needed, stability (not exuding) in the finished article, and the effect on mechanical and barrier properties.

A widely used plasticizer in thermoplastic processing of proteins is glycerol ($C_3H_8O_3$). Glycerol is a low molecular weight, hydrophilic plasticizer that can easily insert itself within the 3-dimensional biopolymer network (di Gioia & Guilbert, 1999). Glycerol is a water soluble, polar, nonvolatile, protein miscible, high boiling point plasticizer making it a suitable plasticizer for use with a compatible water soluble polymer (Gounga, Xu, & Wang, 2007).

Water is the most effective plasticizer in biopolymer materials, enabling glass transition at lower temperatures and facilitating deformation in processing (Hernandez-Izquierdo & Krochta, 2008). However, an excessive amount of water during protein extrusion could decrease melt viscosity and lead to lower specific mechanical energy input. This condition could result in lower product temperature with reduced degree of protein interactions producing poorly formed materials (Hernandez-Izquierdo & Krochta, 2008). Typically dry processes like extrusion and injection molding require that water

content be controlled to avoid formation of pinholes or trapped gas bubbles caused by rapid vaporization of excess water during processing.

Film Formation: Wet vs. Dry Process

For proteins to be used as films for packaging or other applications, they must interact uniformly and form macromolecular networks. Cuq et al., 1998 summarized that formation of macromolecular network from proteins requires three steps: 1) rupture of low-energy intermolecular bonds that stabilize polymers in their native state, 2) arrangement and orientation of polymer chains (shaping), and 3) formation of a three-dimensional network stabilized by new interactions and bonds after the agent that ruptured intermolecular bonds is removed. The two technological processes that are used to make protein-based films are a "wet" process and a "dry" process.

The wet process for protein film formation is based on dispersion or solubilization of protein in solution with casting on a surface to promote removal of the solvent by drying (solvent casting). The functional properties of films obtained by the wet process are highly dependent on several factors: protein concentration in solution, pH, additives, solvent polarity, drying rate, and temperature (Cuq et al., 1998), (Donhowe & Fennema, 1994). The majority of published research on protein and other biopolymer films has been conducted using a solvent cast film method.

The dry process techniques for protein film formation are based on the thermoplastic properties of proteins under low water conditions (extrusion or

compression molding). Protein-based materials can be shaped by extrusion, roller milling, or thermomolding (thermoplastic processing), but process parameters such as temperature, plasticizer concentration, and residence time must be optimized for successful results. To transform proteins using dry processing techniques, it is important to consider the unique behavior of that protein (thermal, mechanical, chemical) above the glass transition with low water concentration. Solubility in water, mechanical properties, and barrier properties of films based on proteins are mainly dependent on the structure of the macromolecular three-dimensional network and on interactions between proteins, plasticizers, and cross-linking agents (Cuq et al., 1998).

Applying standard extrusion techniques that are used to make synthetic polymer films to make sodium caseinate films is an important step toward enhancing commercial use of sodium caseinate films for packaging. Generally, compression molding of sheets is studied as a precursor to extrusion. It is used to demonstrate material flow capability, fusion of components, and help identify processing parameters suitable for extrusion (Sothornvit, Olsen, McHugh, & Krochta, 2007). While most published research on protein-based films has been conducted on solution cast films, utilizing compression molding to produce protein films is the first step toward developing a continuous extrusion process required for industrial polymer processing.

In compression molding, the combination of high temperatures and high pressure (for short times), combined with low moisture content causes the transformation of protein-plasticizer mixtures into viscoelastic melts (Hernandez-Izquierdo & Krochta, 2008). Compression molding can result in the formation of protein-based films with a

range of mechanical and barrier properties that are dependent on the formulation and processing conditions used (Hernandez-Izquierdo & Krochta, 2008). Sothornvit et al. (2003), determined that the use of higher temperatures for compression molding of whey protein films promoted a more extensive protein denaturation resulting in higher cross-linking and reduced solubility.

Research on compression-molded sodium caseinate film is scarce. Arvanitoyannis & Biliaderis, (1998) reported on the mechanical, thermal, gas and water permeation properties of sodium caseinate and soluble starch blends plasticized with water and polyols. Blends used for dynamic thermal analysis and mechanical properties were prepared using a single screw extruder followed by hot pressing. With increasing plasticizer content (water, polyols), there was a progressive decrease of glass transition temperature of the blends. All other published research found on sodium caseinate film has been performed on solution cast films with the exception of US Patent 4,076,846, *Protein-Starch Binary Molding Composition and Shaped Articles Obtained Therefor* .

Nakatsuka et al. describe the invention as:

> More particularly, it relates to a protein-starch binary molding composition, a method for manufacturing the same, and shaped articles obtained therefrom, to which composition can be applied various customary dry processes for molding customary plastics, such as compression molding, transfer molding, injection molding, vacuum forming, pressure forming, etc.
>
> An object of this invention is to provide a so-called water-soluble, edible, thermoplastic molding composition comprising an alkali metal or alkaline earth metal salt of a protein material, a starch material, water, an organic low molecular weight plasticizer, and a lubricant, all of these components being edible.

Sodium caseinate was preferred by the inventors of the patent for its ability to disperse in water, its film-forming property, and its edibility (Nakatsuka et al., 1978).

Also, an edible modified starch, such as methylcellulose, was recommended to improve processability of the composition and physical properties of the sheet and film. Glycerol was listed as a potential plasticizer (depending on sufficient compatibility with the protein) and as a lubricant. Calcium salt was suggested to improve water-resistance. While performing research at Clemson University, Wiles derived a formula from the patent by Nakatsuka et al. (1978) that demonstrated thermoplastic behavior and flow properties suitable for use as compression-molded films (Wiles, 2009).

Protein-methycellulose Blends

The combination of protein with polysaccharides is an effective method in improving the performance of films due to their variability in physical properties and/or their interactions (Gounga et al., 2007). Several approaches have shown the use of proteins with film-forming polysaccharide materials to strengthen barrier properties of protein-based films (Gounga et al., 2007).

Cellulose, the principle structural component of plants, is one of the most plentiful renewable resources and its derivatives are capable of creating tough and flexible edible films with good barriers to aroma, oxygen and oil (Erdohan & Turhan, 2005). As the least hydrophilic water-soluble cellulose derivative, methylcellulose (MC) has been used to produce moisture-sensitive films with a relatively lower water vapor permeability compared with other hydrophilic edible films (Erdohan & Turhan, 2005)

Erdohan and Turhan (2005) also found that tensile strength and percent elongation increased with increasing methylcellulose concentration in methylcellulose-whey protein films.

Addition of Calcium Chloride

Casein interactions with calcium ions and calcium salts (colloidal calcium phosphate) are necessary for formation and stability of casein micelles (Swaisgood, 1993). As discussed previously, the colloidal calcium phosphate is removed by precipitation of the casein at pH 4.6 after which the precipitated casein can be re-solubilized by increasing pH with NaOH, (to obtain sodium caseinate), or CaOH, (to obtain calcium caseinate). The hydrophilic nature of casein and caseinate based films limits their moisture barrier ability when compared to some commonly used synthetic plastic films. Studies by Chen, 2000 and also by Fabra et al. 2010 reported that calcium caseinates improve water vapor barrier properties of caseinate films but impart a more rigid structure. The increase in hydrophobicity could be attributed to divalent calcium cations which promote cross-linking between the negatively charged carboxyl group of the polypeptide chain, thus preventing those charge sites from interacting with water, rendering a more rigid structure (Fabra et al., 2008). Olivas and Barbosa-Canovas, (2008) observed that alginate (carbohydrate-based) films immersed in $CaCl_2$ resulted in films with better barrier properties to water vapor.

Thermal Transitions in Protein Films

The functional properties of a film not only depend on the individual molecular structures and characteristics of the components, but on the combined interactions and thermal transitions of polymers during thermal processing. Proteins are natural polymers that are able to form amorphous three-dimensional structures (Cuq et al., 1998). During heat processing, proteins dissociate, unravel, disaggregate, and denature allowing the molecules to recombine, cross-link, and align in the direction of the flow (Arêas, 1992). Cross-linking reactions lead to high glass transition temperature and high melt viscosity, which is a strong resistance to flow (Hernandez-Izquierdo & Krochta, 2008). However, cross-linking between protein chains also results in more rigid films with better water vapor barrier properties (Fabra, Talens, & Chiralt, 2010).

Glass transition temperature is the temperature range specific to a given material at which the material begins to soften due to mobility of the molecules. Above the glass transition temperature, the plasticized protein becomes soft and rubbery and can be shaped into a desired form. Upon cooling, the matrix network becomes fixed and the material hardens into the imposed form. One complication with thermal processing of proteins is that thermal degradation can occur before reaching the glass transition point. Lower glass transition temperatures are desirable so that less degradation of protein occurs during softening and forming. Plasticizers are used to interact with protein chains to facilitate molecular motion at lower temperatures. Functional efficacy of a plasticizer is often estimated by its reduction in the thermal transition temperature of a polymer

(Kim & Ustunol, 2001). Comparing the thermal transitions of the different sodium caseinate based films should provide a better understanding of the role of water and glycerol as plasticizers for the films.

Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) is one of the most widely used techniques for characterizing the thermal transitions of a polymer. One DSC technique uses an energy input into a polymer sample and an empty reference pan to be measured as a function of temperature. Under a controlled temperature program, the computer measures the difference in the amount of heat required to keep the temperature of the pans increasing at the same rate. Figure 2.2 is an idealized DSC plot that shows the thermal transitions of a semi-crystalline polymer.

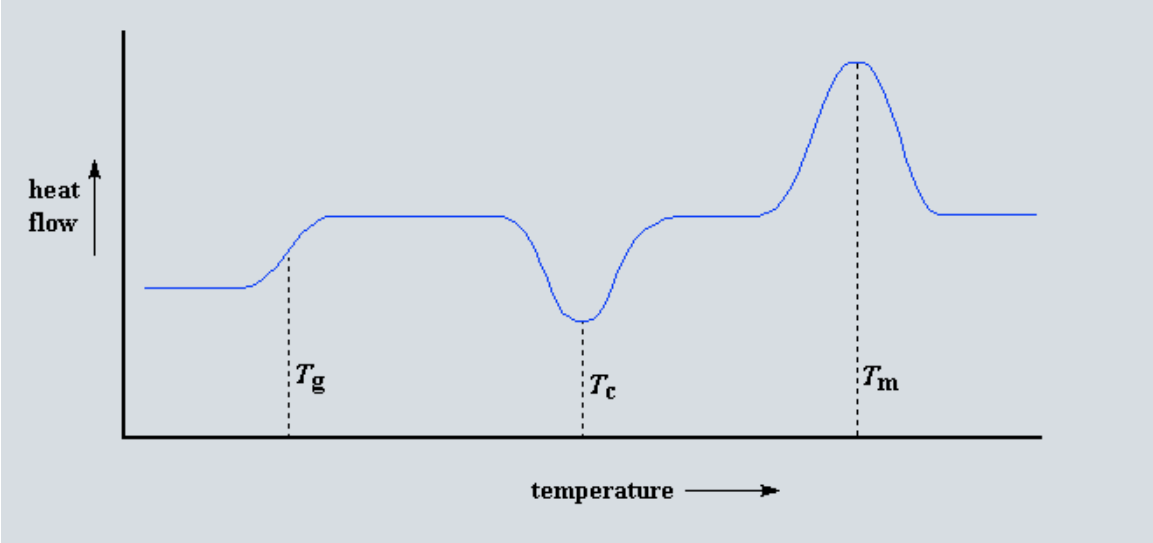

Figure 2.2 Idealized DSC Plot (http://pslc.ws/mactest/dsc.htm)

T_g is the polymer's glass transition temperature. This temperature, which is characteristic of amorphous polymer, is significant because above T_g the polymer is soft and pliable and below this temperature the polymer becomes hard and brittle. DSC can be used to measure a polymer's glass transition temperature because polymers have a higher heat capacity above the glass transition temperature than they do below it (demonstrated by the increase in slope) (Department of Polymer Science, University of Southern Mississippi, 2005). Heat capacity is the amount of heat required to raise the temperature of one gram of matter one degree Celsius. The glass transition doesn't occur suddenly but over a temperature range so the midpoint temperature (middle of the incline as shown by the T_g in Figure 2.2) is most commonly used as the glass transition (ASTM E1356-08).

T_c is the polymer's crystallization temperature, the temperature at which the polymer chains move into ordered arrangements (called crystals), causing a measurable drop in heat flow. The lowest point of the crystallization peak is considered to be the polymer's crystallization temperature (http://www.pslc.ws/mactest/dsc.htm).

Heat may allow crystals to form in a polymer, depending on polymer type and processing, but if heated above T_c, eventually another transition occurs. At the polymer's melting temperature, T_m, the crystals fall apart and the polymer chains become a disordered liquid. A peak on the DSC plot depicts the increase in heat flow required to melt the crystals. The apex of the peak is considered to be the polymer's melting temperature, T_m (http://www.pslc.ws/mactest/dsc.htm).

The crystallization and the melting peaks will only be present in DSC plots of polymers that form crystals. Completely amorphous polymers do not crystallize and therefore do not show T_c or T_m transitions. The presence or absence of a crystallization peak (T_c) is dependent on the polymer's properties and on processing of the polymer.

Thermomechanical Analysis

Thermomechanical analysis (TMA) is another thermal analysis technique that provides a means of detecting changes in hardness or linear expansion associated with the glass transition of polymers (ASTM E1545-05). According to ASTM E1545-05, the polymer sample is placed on a rigid specimen holder while a rigid circular expansion probe, 2 to 6 mm in diameter, contacts the specimen with an applied compressive force of 0 to 5mN. The thermomechanical analyzer measures the change in dimension of a polymer as an observed movement of the sensing probe in direct contact with the specimen as it is heated at a constant rate through the glass transition. The occurrence of an abrupt change in the slope of the linear thermal expansion indicates a transition of the material. The intersection of the extrapolation of the slope of the probe displacement curve before and after the transition is used to determine the glass transition temperature (ASTM E1545-05).

Thermal analysis techniques facilitate the evaluation of glass transition and other thermal transitions in compression molded films. Investigating thermal transitions of sodium caseinate/methyl cellulose films could lead to determination of potential

processing temperatures, heat seal parameters, and prediction of film performance under different end-use conditions ranging from freezing to cooking.

Heat-Sealing Properties

From the observed thermoplastic behavior of sodium caseinate/ methylcellulose films, it was hypothesized that with the correct time, temperature and pressure applied that the films would seal to themselves. Thermal transition temperatures determined by differential scanning calorimetry (DSC) and thermomechanical analysis (TMA) are typically used in determining sealing temperatures of polymers (Hernandez, 1997).

External pressure is needed to bring the sealing layers in contact with each other, while heat is applied, for measured amount of time which is referred to as dwell time. Sealing pressure is the force per area applied to the material by the sealing jaws. A good seal is obtained when enough molecular entanglement has taken place within the polymer chains of the two thermoplastic heat sealing layers to produce a homogenous single layer after cooling (Selke, Culter, & Hernandez, 2004). Measurement of heat-seal strength is the force per unit width of seal required to peel the seal apart after cooling to ambient temperature and is typically used as an indicator of seal quality (ASTM Test Method F 88).

While maximum load force/width is the common seal strength used for comparison, it is of critical importance for practical application to know the mode of seal failure. For example, in food packaging applications it is often desirable to have an easy

opening package where the failure under stress occurs at the seal interface. However, some seals need to be as strong as (or stronger than) the rest of the package to endure the rigors of distribution. The intent of the seal strength test is to measure the force required to peel a seal apart. However, the pulling process may cause another mode of failure. A break or tear of material along the edge of the seal or remote from the seal, as well any elongation of the material, are strength failures of the material itself and must be identified as such for proper evaluation. Seal separation modes on which seal strengths should be reported are adhesive peel, cohesive peel, or delamination as illustrated in Figure 2.3.

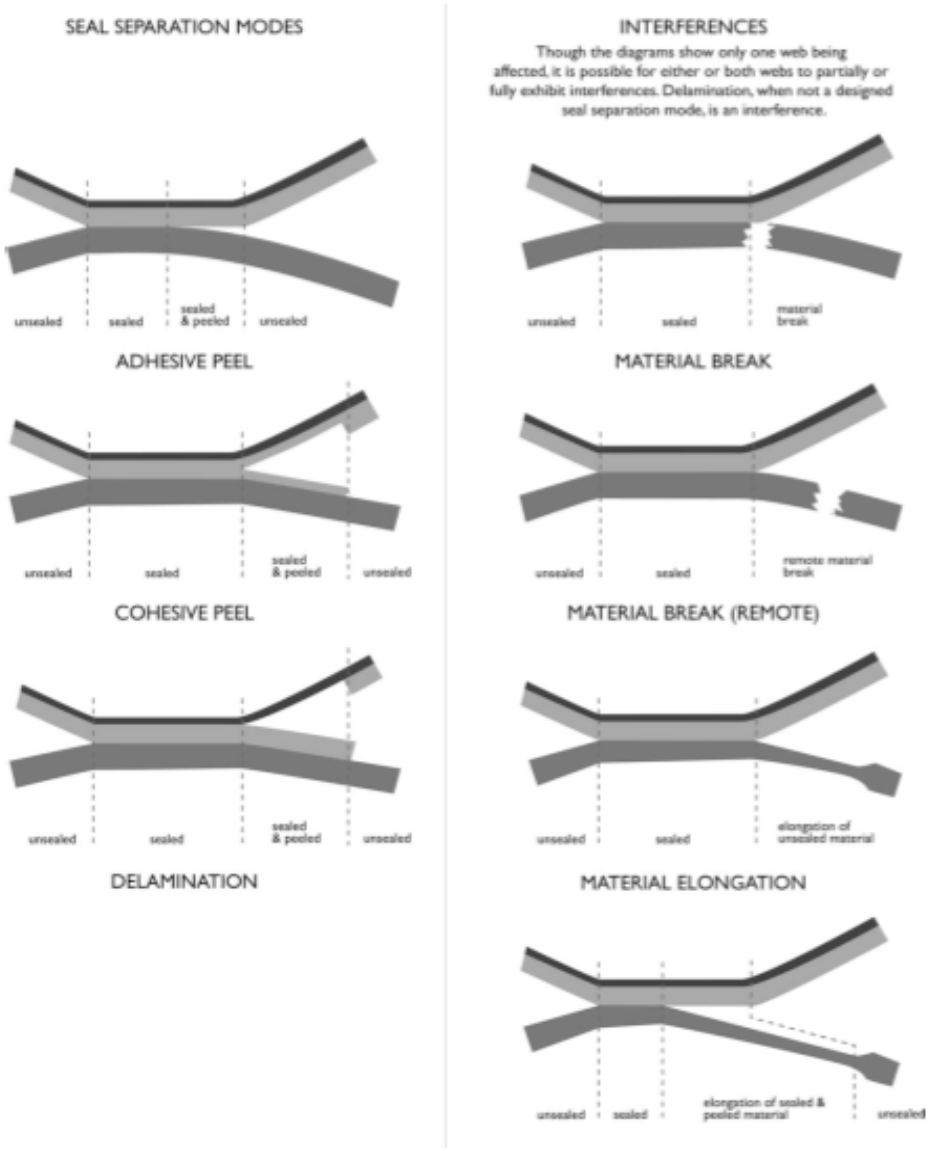

Fig. 2.3 Mode of Failure for Seal Strength Testing (ASTM Test Standard F 88)

Hydration Properties

In the evaluation of biopolymers for use in food and non-food applications like packaging films, some of the most important functional criteria depend on interactions with water; e.g., hydration, swelling, viscosity, gelation, and water holding. In protein-based film formulations, characterizing the water-protein interactions can possibly help explain the role of water in the resin during thermal processing. How water associates with protein can be studied by thermodynamic, kinetic, spectroscopic and diffraction techniques (Kinsella, 1984). Of the thermodynamic methods, the water sorption-desorption isotherm technique is the simplest.

Moisture Sorption Isotherms

Moisture sorption isotherms have been extensively used to describe the relationship between moisture content (wt/wt, dry basis) and water activity (A_w) of biological materials (Khwaldia, Banon, Desobry, & Hardy, 2004), (Kristo & Biliaderis, 2006), (Fabra et al., 2010). Moisture sorption isotherms graphically display the degree of hydration as a function of water activity (A_w). An increase in A_w is almost always accompanied by an increase in the water content, but in a nonlinear fashion. When protein is exposed to increasing equilibrium relative humidity or water activity, a sigmoidal sorption isotherm is obtained that reflects the progressive increase in the

amount of water associated the protein (Kinsella, 1984). The amount of water bound by a protein depends upon its composition, the number of exposed polar groups, surface polarity, pH, salts, and concentration (Kinsella, 1984). When blending proteins with other components for film forming, the sorption behavior of the material becomes much more complex and must be determined experimentally.

There are three types of isotherm curves; adsorption (starting from the dry state), desorption (starting from the wet state), or working (native state) (Decagon Devices, 2004). An isotherm determined by adsorption will not necessarily be the same as one determined by desorption. One way to obtain a moisture sorption isotherm is by placing the sample material into controlled humidity chambers at constant temperature and measuring the sample weight at equilibrium. Containers with saturated salt solutions can be used to create six to nine different controlled water activity levels (Decagon Devices, 2004). The moisture sorption isotherm is presented in either graphical form, by plotting moisture content on the Y-axis as a function of A_w on the X-axis, or as an equation. Studying the moisture sorption of protein-based resins is helpful for understanding how water interacts with the components and its potential role during thermal processing.

CHAPTER 3

MATERIALS AND METHODS

Objective

The objective of the following experiments was to characterize and compare compression molded sodium caseinate/methylcellulose films containing different ratios of water and glycerol as plasticizers.

Raw Materials

Sodium caseinate (NaCas) (C8654) and methylcellulose (MC) (approximate molecular weight 17,000) powders were purchased from Sigma-Aldrich Company, St. Louis, MO. Calcium chloride ($CaCl_2$) powder and glycerol (Gly) were obtained from Fisher Scientific, Fair Lawn, NJ.

Resin Preparation

Dry powders were weighed, stirred with metal spatula, and then mixed in Cuisinart Pro Custom 11 Food Processer (Model DLC-8S) for 30 seconds. Sides, bottom and top cover of the mixing bowl were scraped with a rubber spatula to reincorporate loose material back into the mixture. Distilled water was heated to 85°C and added to

the powder mixture while processing for 1 minute. Again, top cover, sides, and bottom of bowl were scraped with a rubber spatula. Glycerol was added to mixture while processing for 1 minute. After repeating scraping of top cover, bottom and sides of mixing bowl, the resin was mixed for an additional 10 seconds. The formulated resin was weighed and placed in aluminum foil that was folded around resin to form an airtight packet.

The procedure described above was used to make three different resin formulations for compression-molded films. All formulations contained the same amounts of NaCas, MC, and $CaCl_2$. The ratio of water to glycerol was varied while keeping total plasticizer percentage the same.

The three film formulations that were tested were composed of the following materials (Table 3.1) :

Table 3.1 Resin Formulas

OR FORMULA*	**HW (High water)**	**HG (High Glycerol)**
40% NaCas	40% NaCas	40% NaCas
10% MC	10% MC	10% MC
1% $CaCl_2$	1% $CaCl_2$	1% CaCl2
20% distilled H_2O	30% distilled H_2O	10% distilled H_2O
29% Glycerol	19% Glycerol	39% Glycerol

**Original formula from derived U.S. Patent 4,076,846*

Percentages based on weight/total weight

Moisture Sorption Analysis of Resins

Each resin formula was tested for total moisture content and characterized by a moisture sorption isotherm procedure adapted from T. Labuza (1989). Total moisture content was determined by placing approximately 0.5g of resin in an aluminum sample pan, which was then placed in a laboratory oven (LR270, Grieve Corporation, Chicago, IL) at 60°C. Samples were tested in triplicate. After seven days in the oven, the sample pans were cooled to ambient in a glass dessicator. Samples were reweighed and moisture content was calculated on a dry weight basis.

A moisture sorption experiment was conducted on each resin in Table 3.1, in triplicate at 7 different humidity levels. Seven pre-weighed sample cups were filled with approximately 0.1 g of mixed resin. The cups were placed in individual salt solution chambers (Nalgene® cups with lids) that contained equilibrated salt solutions corresponding to seven different water activities as listed in Table 3.2. The salt solution chambers were prepared and equilibrated at room temperature several weeks before testing. The resin samples remained in the salt solution chambers for 1 week at room temperature and then were re-weighed. The sorption tests were conducted on one resin at a time.

Table 3.2 Salt Solutions

Water Activity	**Substance**
0.010	Drierite
0.225	Potassium Acetate
0.428	Potassium Carbonate
0.540	Magnesium Nitrate
0.750	Sodium Chloride
0.850	Potassium Chloride
0.920	Potassium Nitrate

Isotherm curves were determined by comparing the initial sample weights with the weights measured after one week in the salt solution chambers according to the following calculations:

Initial weight (g) – 7day weight (g) = g water lost or gained

Initial weight (g) x % moisture content = g water initially present

Initial weight (g) – g water initially present = g solids initially present

g water initially present – g water lost = g water left (absolute value)

The isotherm curve was plotted using **g water left/g solids initially present** on the Y-axis with **water activities** on the X-axis.

Film Making

The resin was pushed by hand through a brass U.S. Standard No. 20 sieve to assure a common maximum particle size. For OR and HW formulations, approximately 10 g of resin was spread evenly on release paper (silicone coated Kraft paper) to form a 6 cm diameter circle. The paper containing the resin was placed between two steel platens on a custom made hydraulic press (unheated) and "cold" pressed for 1 minute at 1500 PSI. The HG resin was not cold pressed as previously described because it caused the glycerol to squeeze out of the resin, thus preventing proper comparison to the other formulas. Instead, HG resin was sieved one time and then separated into the amounts of 5.84 g and prepared for hot pressing.

The cold pressed resin sheets of Original and HW formulations were sieved using US Standard No. 20 sieve. Sieved resins were weighed into 5.84 g amounts that were spread evenly into 6 cm diameter circles between sheets of release paper. The resin was warmed for 1 minute and pressed with 2500 lbs. force for 3 minutes on a Carver Heat Press (Hydraulic Unit Model #3925, Wabash, IN). Temperature of the steel platens ranged from 190- 200°F.

One batch of each of the three resin formulations was mixed, pressed into film, conditioned, and tested each week for 3 consecutive weeks, for a total of 9 batches (Fig. 3.1).

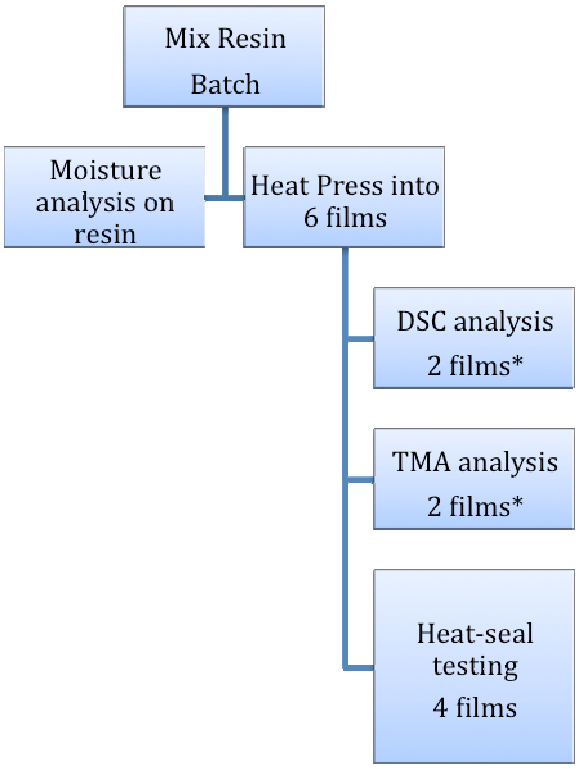

Fig. 3.1 Test Protocol For 3 Resin Formulas

**DMA and TMA tests performed on same film sheets*

Conditioning of Films

All films were conditioned in an environmental chamber (model 9131-3210, Parameter Generation & Control, Black Mountain, NC) for 48 hours at 23° C and 50% RH before measuring and testing.

Thickness

Thickness measurements of the films were made using an electronic micrometer (MS-11C, No.D02604, Nikon Digimicro, Japan). Five measurements were randomly taken on each film. The average of the 5 measurements were recorded for each film.

Thermal Analysis

Thermal analysis techniques were used to analyze changes in the physical properties of the films with changes in temperature. Differential scanning calorimetry (DSC) and thermomechanical analysis (TMA) experiments were performed on two sheets from each batch of conditioned film samples (48 hrs, @ 23°C, 50%RH).

Differential Scanning Calorimetry

Thermal properties were measured using a Differential Scanning Calorimeter (2920 Modulated DSC, TA Instruments, USA). Glass transition temperatures were determined according to ASTM E1356-08. Film samples weighing 7 to 10 mg were cut with a hole-punch, weighed on digital balance (Explorer, OHaus Corporation, Switzerland), and sealed inside aluminum sample pans (TA Instruments, USA). Empty aluminum pans were used as a reference to isolate the heat absorbed by the aluminum

pans. During each run, the DSC cell was flushed with nitrogen at a rate of 20 ml/min and reference pans were empty. The heating rate from 0°C was 10°C/min to 150°C.

Thermomechanical Analysis

The TMA experiment was performed on a TA instruments 2940 Thermomechanical analyzer according to ASTM E1545-05. A quartz expansion probe was lowered onto the surface of a 1cm^2 film sample placed on a quartz stage (Figure 3.2). The force applied by the probe was 0.05N and the temperature ramp was 5°C/min from -5°C to 110°C.

Figure 3.2 TMA Quartz Expansion Probe and Stage (TA Instruments)

DSC and TMA tests were replicated a minimum of 9 times for each formula in a block experiment in which a week is a block. A mixed model analysis of variance (ANOVA) was used with formula as a fixed effect and batch, sheet and interaction of the formula and batch were random effects. DSC results and TMA results were analyzed separately using SAS 9.2 (SAS Institute Inc., Cary, NC). An F-test was used to test for differences.

Heat Seal Testing

From each batch of resin, four of the six films produced were selected for heat seal testing. Heat seal strength was measured according to ASTM Test Method F88 using a universal tensile testing machine (T10000, SATEC Systems Inc, USA). The films were previously pressed into film disks ranging from 19 to 21 cm in diameter and conditioned for 48 hours (23°C, 50% RH). Each film disk was folded in half (on itself) and enclosed in a folded piece of 0.5mil polyester (to prevent sticking to the heat seal bars) (ASTM F 2029-08). Films were sealed using the bar sealer side of a Sentinel Combination Sealer (Model 12-1289 Hyannis, MA). Seal bars were 1 inch wide and covered with TFE - fluorocarbon material. The films were sealed at set point temperatures of 165°F, 175°F, 185°F, and 195°F (sealing jaw temperatures calibrated to within +/- 5 -7°F). Sealing pressure was set for 17.6 psi (jaw pressure 10 psi) and dwell time was 3 seconds.

After cooling to ambient temperature, approximately 2 cm of film were trimmed off either side of the sealed film and the remainder cut into 25mm (1 in) wide strips on a

sample cutter (JDC 1-10, Thwing Albert Instruments) for seal strength testing. All cuts were made perpendicular to the seal. Sealed strips, still supported by polyester film, were placed in the pneumatic smooth rubber grips of the universal tester with each leg clamped so that the seal line was perpendicular to the direction of the pull (Fig. 3.3). Rate of grip separation was 8 in/min. and seal strengths were determined according to ASTM Test Method F 88 as described in the literature review.

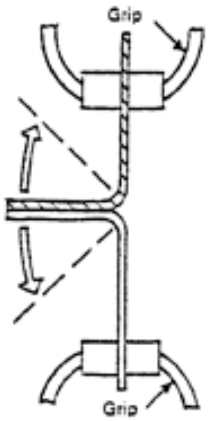

Figure 3.3 Heat Seal Testing Technique (ASTM F88)

The Glimmix procedure in SAS 9.2 was used to analyze the heat seal strength data. In this procedure, a split plot analysis of variance was conducted with formula, temperature, and their interaction viewed as fixed effects. Batch and batch by formula interaction were taken as random effects.

CHAPTER 4

RESULTS AND DISCUSSION

Resin & Film Formation

All of the NaCas/MC film formulations were mixed using the same method as described previously. For objective comparison, all films were heat pressed using the same parameters for temperature, time, and pressure. Visual and physical differences were observed between the compression molded films (Figs. 4.1, 4.2, and 4.3).

HW formulas appeared to be the better films based on objective visual observation of transparency and lack of visible undissolved particles or "specks". They were also the thinnest films (avg. 5.16 mil) compared to OR (avg. 5.60 mil) and HG (avg. 7.57 mil).

The HG formula, with the highest amount of glycerol, produced the thickest films. These films showed clearly defined non-uniform specks of material that had not fully melted and combined with the rest of the components. The specks were not as evident along the thinner outer edge of the films. Specks were also noticeable in the OR formula films although the specks were cloudy in appearance and evenly distributed throughout the film.

Fig. 4.1 OR Formula Compression Molded Film

Fig 4.2 HW Formula Compression Molded Film

Fig. 4.3 HG Formula Compression Molded Film

Visual observations of the films showed that varying the water to glycerol ratio affected the compression molding of the film components. The most notable difference in comparison of the pressed films was the amount of visible specks of unincorporated resin components. Water concentration was inversely proportional to glycerol in the resin formulas and this was a determining factor in the amount of specks as well as their appearance in each pressed film. HW formula, with the highest percentage of water (30%) and least amount of glycerol (19%), produced films where there were no visible specks of unincorporated matter. OR films with next highest amount of water (20%) and with 29% glycerol, showed specks but they appeared cloudy as if not completely dissolved during mixing or compression molding process. Finally, HG formula with 10% and 39% glycerol resulted in compressed films with the most specks with clearly defined non-uniform shape.

From preliminary testing it was determined that water had to be mixed into the formulas before glycerol was added for sufficient wetting of the dry powders to occur with the chosen amount of water in the OR formula (Appendix A). Glycerol and other polyol plasticizers have great water binding capacity (Fabra et al., 2010). Khwaldia et al. (2004) concluded that in NaCas /distilled water dispersions, glycerol interacts with the NaCas chain and water molecules through hydrogen bonding. This leads to the disruption of the protein-protein and protein- solvent interactions. Therefore, it could be that because the glycerol competes for the small amount of water available and reduces the protein-water interactions so that the sodium caseinate could not fully dissolve.

Higher amounts of glycerol produced thicker films. Thicker films in compression molding can be caused by higher viscosity melts. Higher glycerol concentration increases the viscosity and heat capacity of the melt due to the increase in total solids (Khwaldia, Banon, Perez et al., 2004). Siew et al. (1999) attribute the viscosity increase with glycerol content in NaCas solutions to the formation of larger NaCas-glycerol aggregates as glycerol molecules interact with the NaCAS chain. According to that theory, the specks that were seen in the HG films could be large NaCas-glycerol aggregates that are also contributing to greater thickness measurements in these films.

Moisture Isotherm Curves of NaCas Resin

In order to further characterize the resins and to gain more understanding about the role of water in the formulas, a moisture isotherm study was performed. Moisture content of the resin after 7 days of storage was plotted as a function of A_w (Fig. 4.4).

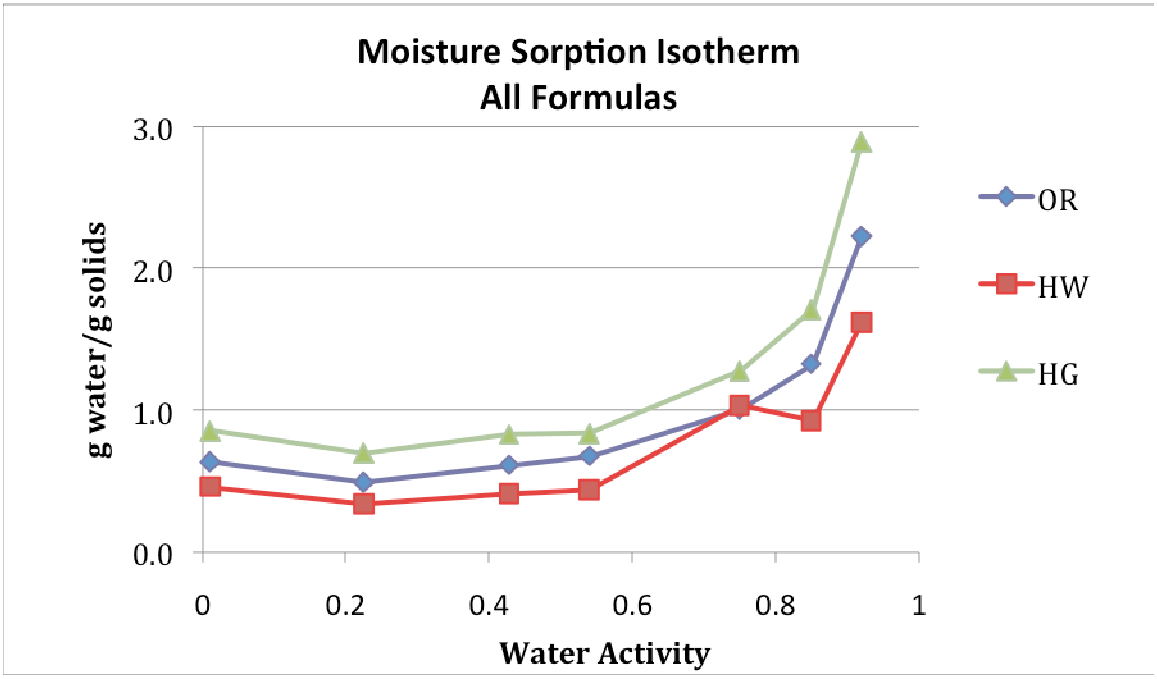

Fig. 4.4 Moisture Sorption Isotherm For All Resin Formulas
Each point represents three replicates. All standard deviations <0.11

The sorption curves of each NaCas-based resin have a shape characteristic of water-vapor sensitive polymers (Khwaldia, Banon, Desobry et al., 2004). The sharp increase of moisture content with increasing water activity indicates that the solubility of water in the polymer varies with the partial pressure of water vapor. These results were as expected because sodium caseinate is characterized by high water adsorption (Southward, 1985). Similar isotherms were reported by Fabra et al. (2010) for solution cast sodium caseinate films with glycerol (Fig. 4.5).

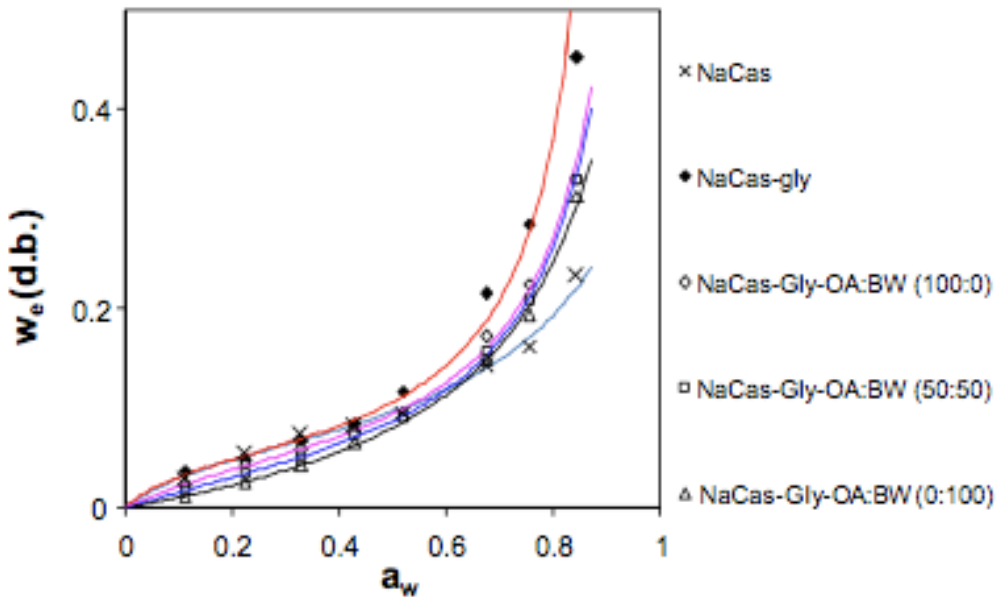

Fig. 4.5 Sorption Isotherms For Solution Cast NaCas-Gly Films (Fabra et al. 2010)

In comparison of the curves produced by the different formulas in the current study, hydration slightly increased with increasing concentration of glycerol (HW 19%, OR 29%, HG 39%). The increasing moisture affinity of protein-based films with increasing plasticizer concentration has been documented in the findings of Kristo and Biliaderis (2006) for glycerol-plasticized pullulan and sodium caseinate films and their blends, by Mahmoud and Savello (1992) for glycerol-plasticized whey protein films, and by Cho and Rhee (2002) for sorbitol and /or glycerol-plasticized soy protein film. Researchers have concluded that the plasticization and swelling of the polymer matrix as the moisture content of the film increases, provides more binding sites for water (Khwaldia, Banon, Desobry et al., 2004). As discussed previously, glycerol is highly water binding so the isotherms confirm that the formulas with more glycerol have potential to absorb more water at higher water vapor pressures.

Characterization by Thermal Analysis

Thermal analysis was used to further characterize the role of water and glycerol as plasticizers for compression molded NaCas/methylcellulose based films. Differential scanning calorimetry (DSC) and thermomechanical analysis (TMA) are both commonly used thermal analysis techniques that characterize the glass transition temperature (T_g) for amorphous polymers. Each detects T_g based on changes in a different material property during the glass transition. The material property measured by DSC to detect transition temperatures is heat capacity, while TMA measures expansion coefficient or softening. The relative sensitivities of the different techniques for detecting the T_g vary depending on the nature of the material being evaluated as well as on experimental variables such as the heating rate (Foreman, Sauerbrunn, & Marcozzi,).

Two films from each batch of resin were analyzed for T_g using DSC and TMA with at least 2 replicates/test on each film. Formula, batch, and film number of each sample tested were recorded. The focus of discussion on thermal analysis results will be characterization and comparison of the formulas.

Thermal Analysis by DSC & TMA

DSC thermographs of the three sodium caseinate/ methylcellulose films showed that they were purely amorphous with no crystallization or melting peaks (Fig. 4.6). As

mentioned previously, average transition temperatures were based on formula-to-formula comparison (at least 9 replicates each). In DSC analysis, glass transition temperatures (T_g) are determined by a change in heat capacity of the polymer. T_g in this study was observed as gradual slope in the baseline over a range of temperatures (Fig. 4.7). T_g temperatures were reported as the midpoint of the transition curve (ASTM E13556-08).

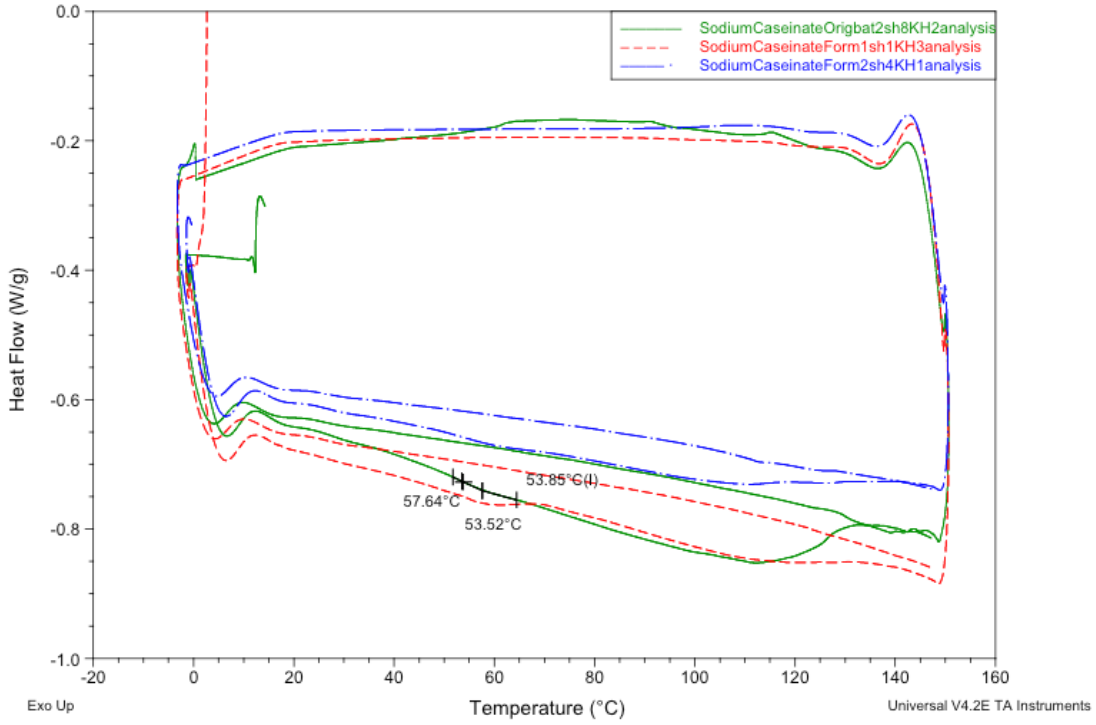

Fig. 4.6 DSC Thermograph Overlay of All 3 Films

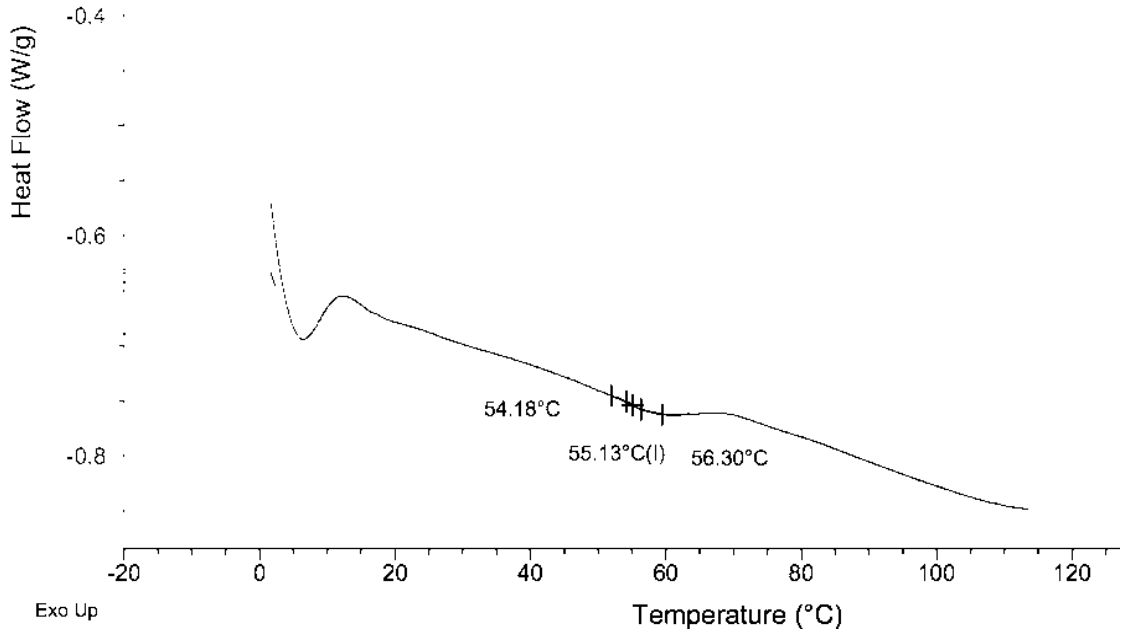

Fig. 4.7 Glass Transition of NaCas/MC Film Determined by DSC

In the present study, DSC analysis of the NaCas/ MC compression molded films with varying water to glycerol ratio, showed the average T_g for all formulas to be between 52.8 and 54.2 °C (Table 4.1). Overlays of DSC thermograms illustrate similarities in the formulas (Fig. 4.6). Statistical analysis showed that there were no significant differences in T_g ($p > 0.05$) between the 3 film formulations based on a minimum of 9 measurements per resin formula.

Table 4.1 Transition Temperatures of NaCas/MC Films

	Transition Temperatures	
Resin Formula	DSC Tg (^oC)	TMA Tg (^oC)
OR	54.2 $\pm$ 1.7	49.4 $\pm$ 5.9
HW	53.2$\pm$ 1.6	48.9 $\pm$ 2.1
HG	52.8 $\pm$ 2.4	49.5 $\pm$ 2.1

TMA testing (minimum 9 measurements each formula) also showed no significant differences in T_g ($p > 0.05$) between the 3 film types (Table 4.2). TMA measurements of T_g were determined using a quartz expansion probe, which measures dimensional changes (softening) in the film as a function of temperature whereas DSC measures changes in heat capacity (Fig. 4.8). As determined by TMA, the T_g was slightly lower than that detected by DSC for all films. The differences between T_g measurements by the two techniques are consistent and acceptable considering the differences in relative sensitivity (relative signal change) of each analysis technique for detecting the T_g (Foreman et al.,).

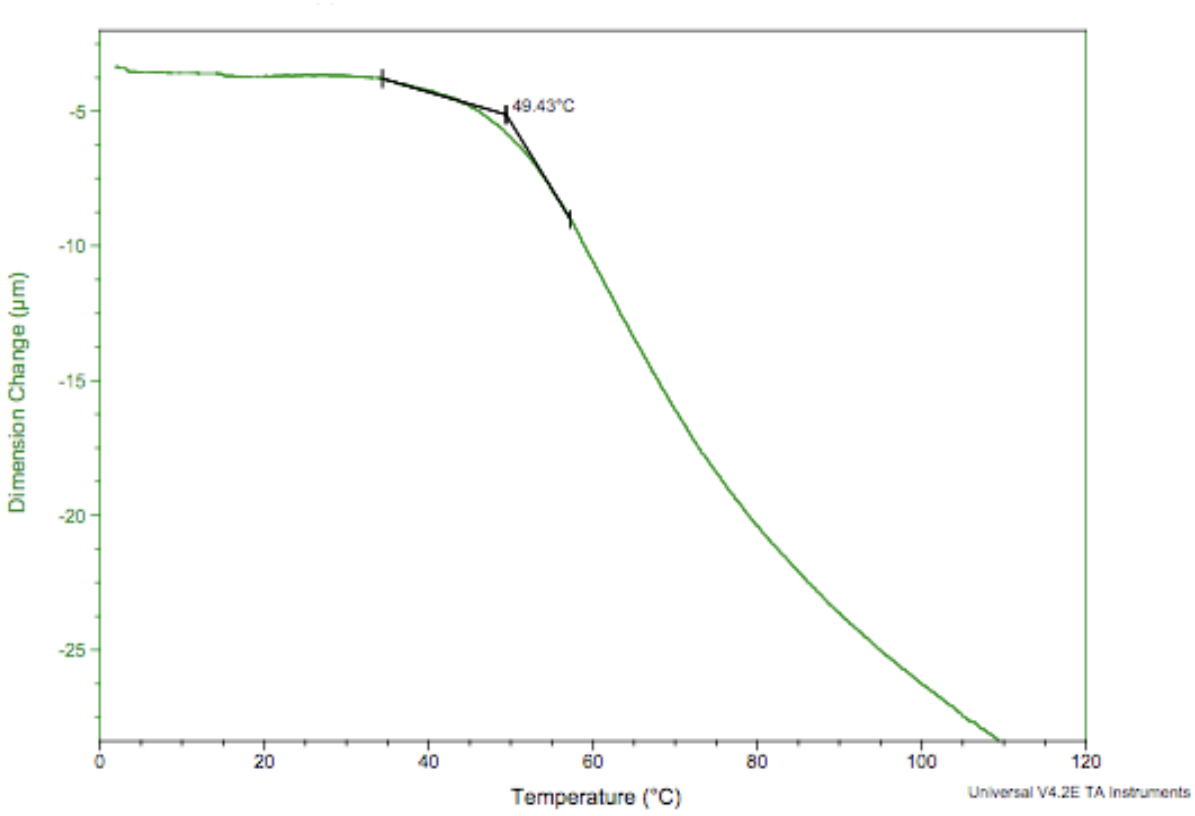

Figure 4.8 Glass transition of NaCas/MC Film Determined by TMA

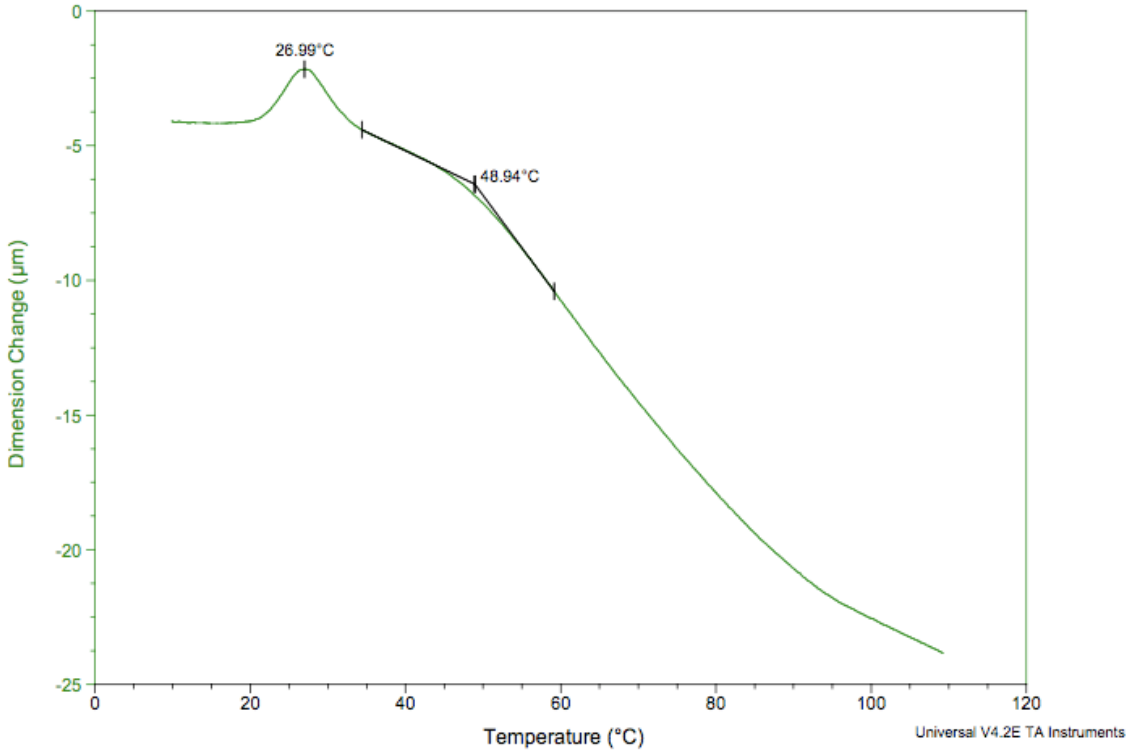

Figure 4.9 Glass transition of NaCas/MC Film Determined by TMA with Transient

As noted by ASTM Standard E1545-05, a transient was observed between the pre-transition slope and the final slope (Fig 4.9). This was observed in the same temperature range (22-28°C) in film samples from all of the formulas. The test method states that this anomaly can occur when testing some materials due to settling, residual stresses within the specimen, or alteration of the specimen morphology. DSC analysis did not show a change in morphology in any of the films in the range where the pre-transition humps appear on the TMA curves. Residual stresses are unlikely to be the cause due to the fact that films were allowed to cool to room temperature without any mechanical or thermal forces being applied. Material settling, although not really well

defined in the ASTM method, is the most likely explanation because it was noted in preliminary testing that the samples were prone to compaction when measuring thickness (Appendix).

Several studies have proven the plasticizing effect of glycerol and/or water on sodium caseinate solution-cast films (Arvanitoyannis & Biliaderis, 1998), (Khwaldia, Banon, Perez et al., 2004), (Siew et al., 1999). In the current study on compression-molded films, the percentage of total plasticizer (water and glycerol) in the resin formulas did not change, only the ratios changed by 10% higher and lower than the OR formula. According to thermal analysis, the ratio of water: glycerol in the resin formulas did not have a notable affect on T_g in these sodium caseinate/methylcellulose based films after conditioning for 48 hours @ 50% RH. From thermal analysis of T_g by DSC and TMA, water and glycerol were equally affective as plasticizers for these compression-molded sodium caseinate/methylcellulose films.

Heat Seal Properties

Film samples from each of the three batches of resin formulas were sealed at 165, 175, 185, and 195°F using the same dwell time and pressure. The strengths of 5 seals at each temperature were measured on a tensile testing machine and the mode of seal failure was determined according to ASTM Test Method F 88.

Seal strength results from batches of each film formula were combined for analysis by film formula (Fig. 4.10). Statistical analysis was conducted on maximum

load force/width (gf/25mm) because that is a measure of the force required to open a sealed package made from these films.

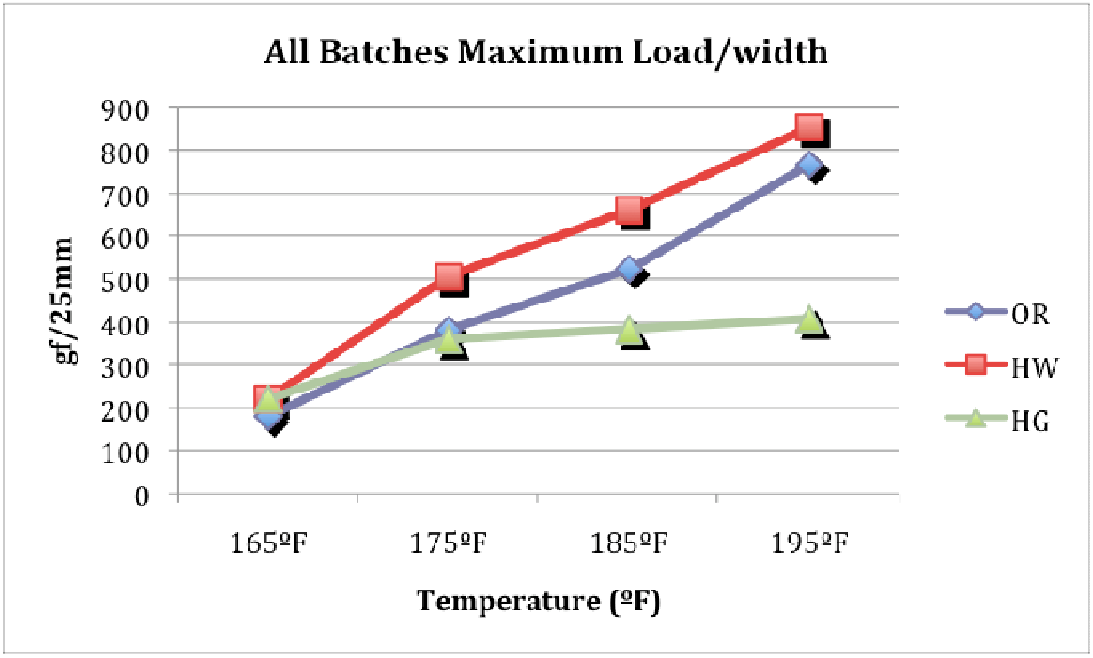

Fig. 4.10 Maximum Heat Seal Strengths for All Films

Preliminary testing showed that seal initiation (noted by seal strengths above 125 gf/25mm) occurred at 165°F for OR films (Appendix A). Since transition temperatures of the films from the 3 formulas were not significantly different, it was expected that all three films had similar seal initiation temperatures so seal strengths at 165°F would be comparable. Statistical analysis confirmed that at 165°F and 175°F there were no significant differences in seal strength between film formulas. However, at seal temperatures of 185°F and 195°F, maximum seal strength for HW seals became statistically higher than HG seals (Table 4.2). At these temperatures, molecular

entanglement became more complex so the force required to disrupt the sealed interface was higher. When films became completely fused, failure of the seal involved destruction of the material. Seal strength as it is being measured at that point becomes a measure of the material's tensile strength instead of adhesion at the seal interface.

Table 4.2 Seal Strengths

	SEAL STRENGTHS (gf/25 mm)		
TEMPERATURE	**OR**	**HW**	**HG**
165°F	180 ± (71)	221 ± (129)	221 ± (101)
175°F	382 ± (125)	506 ± (358)	360 ± (163)
185°F	524 ± (193)	662 ± (149)	383 ± (139)
195°F	768 ± (669)[a]	855 ± (203)[a]	408 ± (84)[b]

a significantly higher than b

Siew et al. (1999) reported a large decrease in tensile strength with increase in glycerol concentration in solution cast NaCas/glycerol films. As mentioned in the literature review, glycerol is an effective plasticizer for protein because it can easily insert itself within the 3-dimensional biopolymer network (di Gioia & Guilbert, 1999). The result is increased mobility in the protein chains and increased elongation of the material under tensile stress. Additionally, the interference of the glycerol with protein-protein bonds results in weaker seals because proteins are unable to entangle as much at the seal interface. Similarly, in the current study, at sealing temperatures of 185°F and 195°F, fusion was beginning to occur and films with more glycerol had lower maximum

load/width seal strengths. This result was due to lower tensile strength and less entanglements at the seal interface compared to OR and HW films.

A break or tear of material along the edge of the seal or remote from the seal, as well any elongation of the material, are strength failures of the material itself and must be identified as such for proper evaluation. Figures 4.11, 4.12, and 4.13 represent seal strengths for each formula displaying average load force/width for seals that peeled apart and maximum load force/width for seals that failed by tearing or breaking. Load forces for each failure type were averaged by film (i.e. some films had seals at the same temperature with both types of failure). No breaks or tearing occurred out of the seal areas (remote).

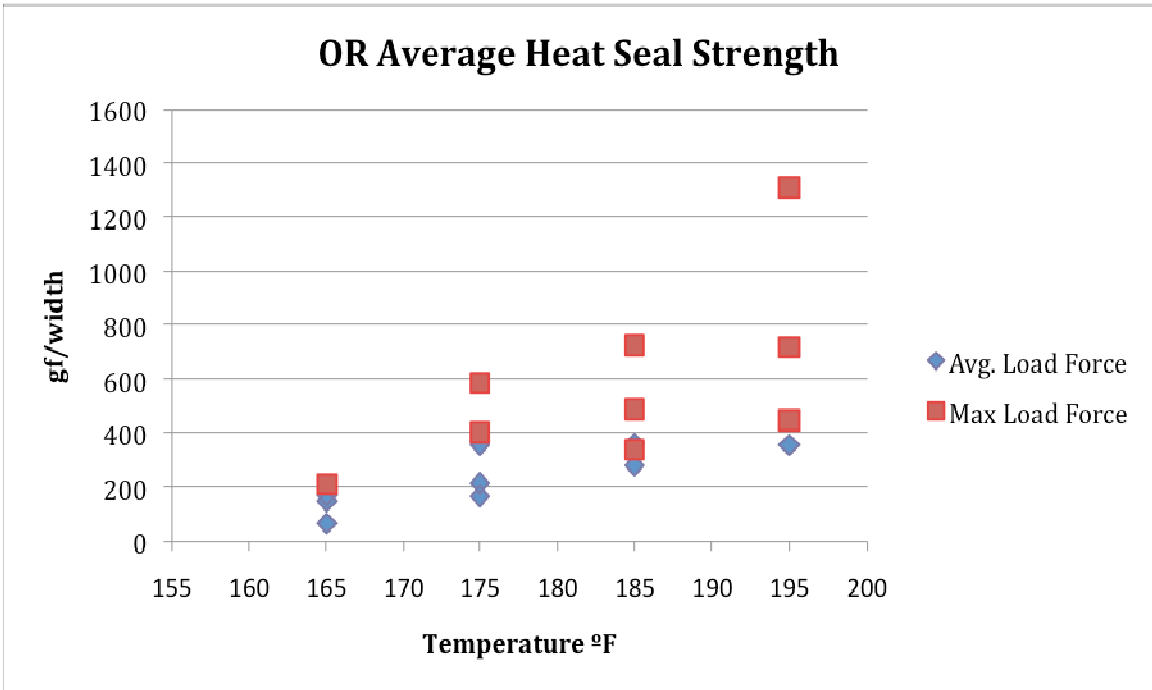

Figure 4.11 OR Heat Seal Strength by Failure Mode

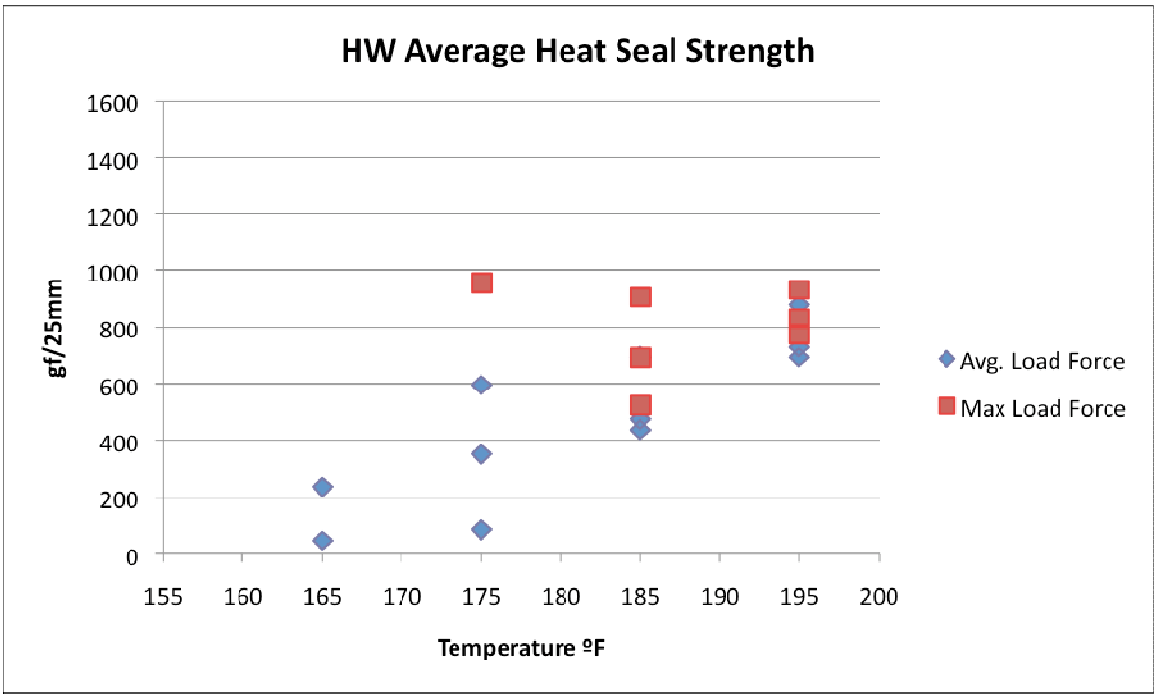

Fig. 4.12 HW Heat Seal Strength by Failure Mode

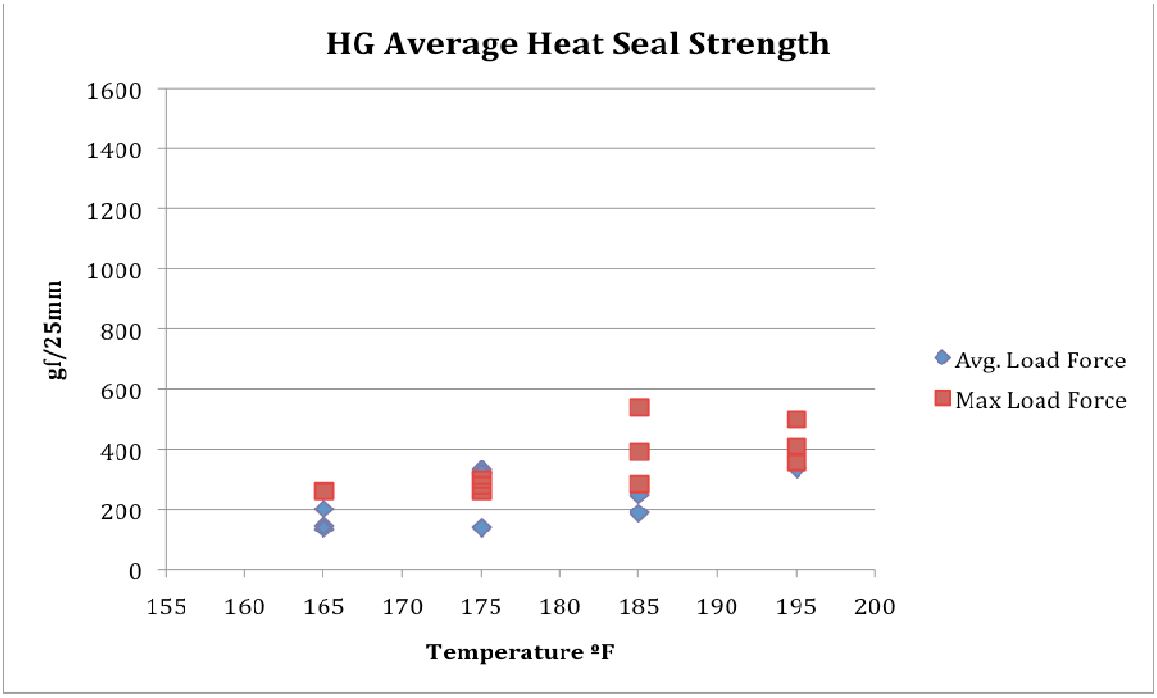

Figure 4.13 HG Heat Seal Strength by Failure Mode

All formulas show higher seal strengths and more destructive seals as sealing temperature increased. Table 4.2 is a numerical compilation of seal failures by type (peel or destructive) for each film at each of the 4 sealing temperatures. Destructive seal failures were experienced at 165°F for HG and OR films. Statistical analysis showed only a formula effect at 185°F and 195°F. Low strength destructive seals at 165°F and 175°F were likely due adhesion created by the pressure of the seal bars due to the tackiness of the films, especially films with higher glycerol content. No failures due to fusion were noted for any films at 165°F and 175°F because the sealed layers could be peeled apart by hand.

Table 4.3 Seal Failures

Seal Temperature	OR	HW	HG
165ºF	12 peel / 3 tear	15 peel	14 peel / 1 tear
175ºF	9 peel / 6 tear	10 peel / 5 tear	12 peel / 3 tear
185ºF	3 peel / 12 tear	6 peel / 9 tear	5 peel / 10 tear
195ºF	1 peel / 3 tear / 11 F	3 peel / 10 tear / 2 F	2 peel / 1 tear/ 11 F

F means completely fused

Completely fused seals, where the break occurred at the seal line and the layers could not be separated, only occurred at seal temperatures of 195°F. From the HG films, only 1out of 15 heat seals were completely fused. These films were the thickest and had the highest amount of glycerol. The HW films had only 2 fused seals. The HW films

needed higher temperatures to completely fuse but they were still the strongest at break (highest max force load/width). OR films had the highest number of fused seals (11/15) and but seal strengths were not as high as HW. Statistically, both OR and HG had seal strengths significantly higher than HG at 195°F ($p < 0.05$). The glycerol content was high enough to cause lower seal strength but not high enough to interfere with protein entanglement in the seal as much as it did in the HG films.

These results show that the seal failure type and strength can be modified by varying the water to glycerol ratio. The OR formula with the most balanced ratio (20% H_2O: 29% Gly) demonstrated the effects of both plasticizers that were discussed previously. Reduced levels of glycerol gave stiffness and strength to the seals while providing mobility the protein chains for lower stresses under tensile strain.

CHAPTER 5

CONCLUSIONS

In this study, compression-molded sodium caseinate based films with different water: glycerol percentages were characterized. Moisture isotherm experiments showed that all formulas were moisture sensitive and that moisture absorption increased with increasing amounts of glycerol at all water activities. From the high water binding property of glycerol that was observed in the moisture sorption experiment, it was theorized that the glycerol competed for the small amount of water in the resin formula preventing the sodium caseinate from being able to fully solubilize during compression molding in formulas with >29% glycerol. Visual inspection of the films confirmed this theory: in HG films (39% glycerol), opaque specks of unincorporated components were highly visible, less visible in OR films (29% glycerol), and not macroscopically visible in any HW films (19%). Additionally, higher amounts of glycerol in HG formulas increased the amount of total solids causing higher viscosity melts which resulted in thicker films formed by compression molding.

Thermal analysis by DSC and TMA showed that all films were amorphous and that water and glycerol were equally effective at lowering T_g within the different water: glycerol ratios that were tested. Similar T_g in the films resulted in similar seal initiation temperatures. Seal strengths (maximum load force/width) for all films were not significantly different at 165 °F or 175°F seal temperatures. However, at a seal

temperature of 195°F where seal fusion began to occur, significantly lower seal strengths were measured for HG films compared to OR and HW films. Lower seal strengths were attributed to lower tensile strength in the HG film caused by increased amounts of glycerol. While water and glycerol were equally effective at lowering T_g, they affected the strength properties of the film in different ways. Glycerol decreased the tensile strength and heat seal strength because it interacted with the protein chains, increasing mobility. Higher concentrations of glycerol increased elongation of the material under tensile stress and interfered with protein entanglements at the seal interface, which resulted in lower seal strength (low stress). Water increased stiffness causing increased film strength and seals with higher breaking stress.

Characterization of compression molded sodium caseinate/methylcellulose based films in this study showed that they could be further developed for commercial use. Results from this research demonstrated that the films are transparent, highly moisture sensitive, and heat sealable. According to the patent, the films are also edible and water-soluble.

Considering the properties of the sodium caseinate compression molded films and the need for sustainable packaging, these films could be used for many applications. The films could be sealed into small pouches of pre-measured protein powders or condiments where the pouch can be directly added to the water-based liquid and then blended, dissolved and consumed without any packaging going into the waste stream. An added benefit may be a thickening affect provided by the sodium caseinate.

Low seal strengths and peelable seals present the possibility of using the films in applications where easy-open seals are needed. Pouches made from these films could be made for dry snack foods in a child's lunchbox. The empty pouch could be put down the garbage disposal or possibly fed to a pet. If research on these films continues, they could be used for a wide variety of environmentally friendly packages.

CHAPTER 6

RECOMMENDATIONS

Recommendations for future research on compression molded sodium caseinate based films include the following:

- Intensive mixing studies should be performed to facilitate homogenization and incorporation of all film components.
- Rheological analysis should be utilized to fully characterize flow properties of the melt and help determine extrusion parameters and possible applications for injection molding.
- Heat-sealing coating of sodium caseinate films to existing films should be further explored.
- Effects of compression molding on film solubility should be studied.
- Effectiveness of other plasticizers for compression molded sodium caseinate film should be analyzed.
- Research on improving mechanical strength and water vapor barrier properties of compression molded sodium caseinate films should be conducted.

Appendix

Preliminary Testing

Preliminary tests were performed on the original resin formula that was derived from U.S. Patent 4,076,846 to determine mixing and pressing parameters. Rheology testing was used as an initial step toward characterization of the resin melt properties. Extreme ratios of water to glycerol (0:100 and 100:0) were pressed into films to verify need for both plasticizers. Optimum press times and temperatures were determined to be able to produce flexible and visually acceptable films that could be handled for characterization experiments. Heat seal testing was performed to determine time, temperature and pressure settings for seal initiation on OR films.

Materials and Methods

Sodium caseinate (NaCas) (C8654) and methylcellulose (MC) (approximate molecular weight 17,000) powders were purchased from Sigma-Aldrich Company, St. Louis, MO. Calcium chloride ($CaCl_2$) powder and glycerol (Gly) were obtained from Fisher Scientific, Fair Lawn, NJ.

Mixing Trials

Initial trials with derived formula involve mixing by hand, which quickly proved to be inadequate for any sizable amount of materials. Next, a Kitchen Aid mixer (K5-A 300 watt) was employed for mixing but the particle sizes in the mixture were not uniform. A Cuisinart food processor (Pro Custom 11) was determined to be the best available equipment for blending the components and producing uniform resin. Also during mixing trials it was noted that water had to be incorporated into the mixture before glycerol or the mixture would require more than double the original amount of water to wet the dry powders. Additionally, optimum mixing times were determined that would not dry out the resin too much during this process, because it resulted in poor quality films that exuded glycerol.

Rheology Testing

Rheology testing was performed by Sam Lukubira, a doctorial candidate in the Department of Chemical Engineering at Clemson University. OR resin was tested using DMA experiments on an ARES II Rheometer (TA Instruments, USA) to try to determine characteristics of the melt flow properties for possible extrusion and/or injection molding if such equipment were accessible. Results of a dynamic sweep test on the resin demonstrated a linear viscoelastic region in the material but further steady state tests at these amplitudes were inconclusive and problematic due to the sticky behavior of the

melt at higher temperatures (Fig.A.1). Rheology testing was discontinued and it is recommended that more work be done on mixing behavior of this material in the future.

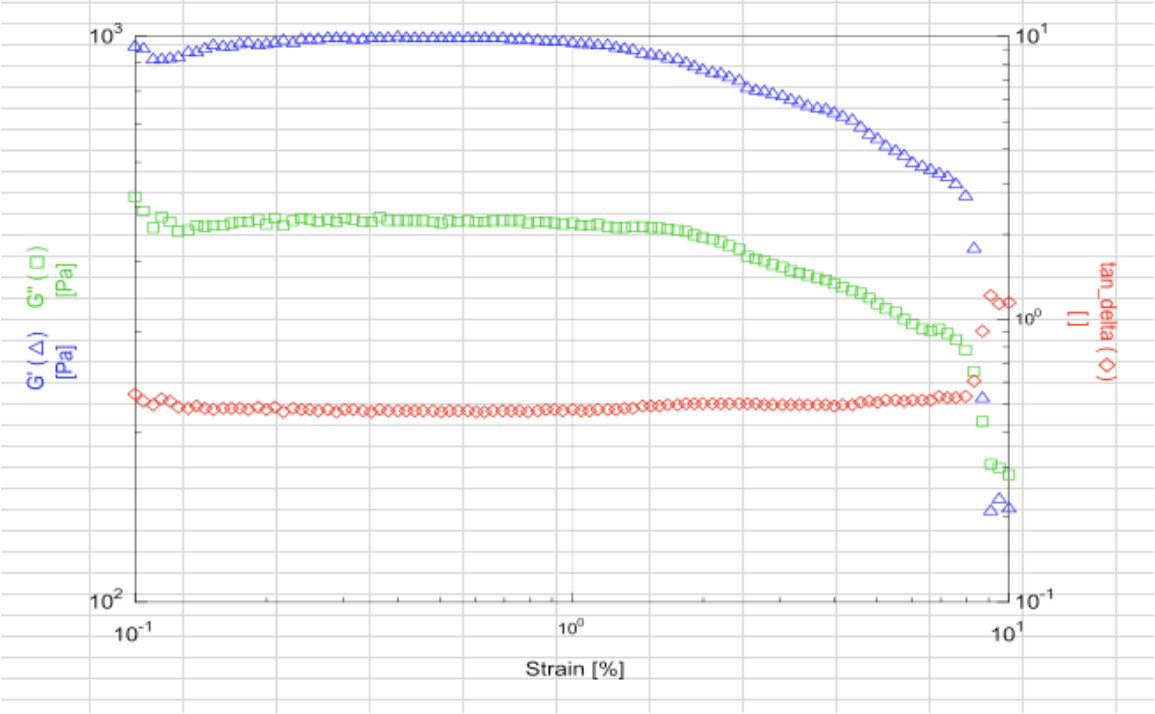

Figure A.1 Dynamic Strain-sweep Test on OR Resin

Effect of Water or Glycerol Elimination

Batches of resin with water to glycerol ratio 0:100 and then 100: 0 were mixed and pressed into films. The 0:100 films exuded glycerol both during and after heat pressing and were too thick, while 100:0 films became brittle and cracked within minutes of cooling to ambient after heat pressing. From these results, it was determined that both plasticizers were necessary for acceptable films. To learn more about how water was

behaving in the resin, a moisture isotherm study was added to the list of characterization tests to be performed.

Compression Molding by Heat Press Trials

Before heat pressing, resins were sieved in U.S. Standard No. 20 sieve to alleviate clumping that occurred during storage of mixed resin and ensure uniform particle size. Cold pressing and sieving a second time were used to provide an added shear to the resin, which seemed to result in films with less specks or clumps of unincorporated material.

Heat pressing was performed on a Carver Heat Press (Hydraulic Unit Model #3925, Wabash, IN) to find time and temperature parameters that would produce transparent and speck-free films derived (OR) formula that were flexible but not so thin that they could not be handled for testing purposes. Temperature range for acceptable films was determined to be 185 - 195°F with warm-up time of 1 minute and pressing for 3 minutes. Press temperatures above 250°F resulted in scorched films with a lace pattern. Films pressed below 175-180°F were too thick (14mil) and had too many visible specks of material. Press time was determined by pressing same amounts of resin for 1,2, or 3 minutes in the middle of the determined temperature range (190°F) to determine which press time produced thin and clear films but did not cause resin to run off platens or scorch. Resin had a strong tendency to stick to platens so silicone release paper was chosen over silicone spray so that films would not be “tainted” with residue from the

spray. Accurate thickness measurements were difficult due the pressure-sensitive nature of the films.

Seal Initiation Determination

OR compression molded films were tested to determine heat-sealability and to determine what dwell times, pressure and temperatures were necessary for seal initiation to occur. To prevent sticking to seal bars, 0.5 mil polyester was used to enclose strips of OR film folded on itself. Seal initiation temperature, according to ASTM F2029, is the sealing temperature at which a heat seal of significant strength (125g/25mm) is produced. This occurred for 5.5 mil OR films at 165°F with seal pressure of 17.6 psi. At 200°F and pressure 17.6 psi, seals showed slight distortion. Pressing conditions for research were chosen within these parameters to create measurable seal strengths.

Results and Discussion

Preliminary testing resulted in the determination of a mixing and resin preparation protocol using a food processor, cold press and U.S. standard No. 20 sieve for uniform, clump-free resins. Optimum heat press conditions and press times were determined to produce films with good transparency and flexibility yet able to be handled for heat seal testing and thermal analysis. Heat seal conditions were chosen to provide measurable

seal strengths. Moisture analysis was added to provide insight on the effect of water in the resin formulas.

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